a new LPS-triggered phenomenon within the cytoplasm. This observation also raises the possibility that regulation of P2X7 sensitivity to ATP might also function in other circumstances and biological systems, characterized by P2X7 engagement in response to apparently low concentrations of ATP.

The mechanism by which P2X7 drives cytotoxicity is also poorly understood and will have to be clarified. It needs to be demonstrated that pore-formation is sufficient to drive membrane disruption upon ATP ligation.

Another question remaining in the field is to understand whether IL-1β release upon inflammasome activation is a passive event that relies on membrane disruption or an active and regulated mechanism. The fact that upon intracellular LPS exposure P2X7 deficient macrophages release IL-1β in absence of pyroptosis suggests that in some circumstances IL-1β release can be part of an active process. The detailed study of P2X7-deficient macrophages might therefore turn out to a valuable approach to study possible IL-1β secretion mechanisms that might occur independently of cell lysis.

Finally, another caspase-11 substrate, Gasdermin D, has been identified recently (Kayagaki et al., 2015; Shi et al., 2015). Its cleaved product has been shown to initiate a pyroptosis program per se. However, how it exerts cytotoxic function remains to be discovered. Future studies will tell us whether this inflammatory caspase substrate co-operates with pannexin-1 and P2X7 to elicit cell death or whether it defines an alternative form of pyroptosis. Similarly, it is unclear whether caspase-1 mediated pyroptosis relies on the same substrates as caspase-11. If distinct substrates trigger different death programs, the question that might arise is whether these subtypes of pyroptosis have different inflammatory properties.

Pyroptosis-mediated inflammation can have different outcomes ranging from healing infections to sepsis and death. Unlocking the mechanisms regulating the initiation of pyroptosis are great steps forward that will bring us on the path to a better understanding of the physiological roles of pyroptosis. This will, in the long run, contribute to the development of therapeutic strategies aimed at enhancing the defenses against pathogens and tumors, or when pyroptosis is too strong, to limit the harmful consequences of inflammation.

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# Common Characteristics of HIV-Neutralizing Antibodies with a Fondness for Sugars

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**Broadly neutralizing antibodies targeting quaternary epitopes on the apex of the HIV-1 envelope spike are an attractive vaccine target, yet engineering immunogens that recapitulate such epitopes has proven difficult. In this issue of *Immunity*, Andrabi and colleagues (2015) identify an exciting new candidate immunogen that could initiate the production of these types of antibodies through vaccination.**

After several years of HIV-1 infection, subsets of individuals develop broadly neutralizing antibodies (bnAbs). Advances in antigen-specific B cell isolation and single-cell antibody cloning have led to the characterization of an increasing number of monoclonal antibodies that can potently neutralize a wide range of HIV-1 strains from such subjects. Passively delivered bnAbs protect against HIV-1 infection in experimental animal models, and it is expected that if elicited by vaccination, they could provide sterilizing immunity in humans. bnAbs target four major sites on the HIV-1 envelope

glycoprotein (Env) spike, one located at the base of subunit gp41 and three located in subunit gp120: the CD4 binding site (CD4-BS), a region centered around a conserved glycan at position 332 at the base of the third hypervariable region (V3), and a region made up of the first and second Env hypervariable regions (V1 and V2) at the trimer apex (Burton and Mascola, 2015). Recently, bnAbs whose epitopes span gp120 and gp41 were identified. bnAbs that target the same sites of Env and have similar structural features are grouped into classes. Understanding the structural features and ontogenies of the different class members not only provides insight into how the human immune system responds to infection but also can assist immunogen-design efforts aimed at eliciting antibodies that target these vulnerable sites on Env through vaccination.

In this issue of *Immunity*, the study by Andrabi et al. (2015) provides important new information on bnAbs that target the Env spike's apex, where the V1 and V2 regions of the three protomers forming the spike come together to form a canopy. These "V2 apex bnAbs" are attractive targets for vaccine development because (1) they arise in ~20%–40% of individuals who develop bNAbs, demonstrating that their epitopes are immunogenic and thus could be elicited in a significant proportion of vaccinated individuals; (2) they are not as mutated as other types of bnAbs, so their elicitation might not require complex and prolonged immunization regimens; and (3) they emerge sooner in infection than other types of bnAbs. However, there has been no clear strategy to elicit V2 apex bnAbs by immunization. In this study, the authors examine the binding and neutralizing activities of four prototypic V2 apex bnAbs (PG9, CH01, PGT145, and CAP256.09) isolated from different donors in an effort to identify common features that allow these antibodies to neutralize a diverse range of HIV-1 strains despite binding to a highly variable region of Env.

At the V1-V2 apex, these four prototypic bnAbs home in on a lysine-rich region that is largely shielded by conserved glycans. Although the extensive glycosylation of Env is thought to limit its recognition by antibodies, V2 apex bnAbs have found ways to take advantage of this sugar coating. Because of their unusually long (≥24 aa) CDRH3 regions (9–11 aa in most antibodies), V2 apex bnAbs are able to penetrate the glycan shield. In doing so, they also make direct contact with a wide range of diverse sugars at positions 160 and 156.

Although the four V2 apex bnAbs examined target Env in similar ways, they each evolved independently in different individuals and have distinct ontogenies. Two, PG9 and CAP256.09, are derived from different VH genes, yet they share 99% VH sequence identity. They also share a D-gene-encoded motif in their CDRH3; this region is important for binding and neutralization. The other two, CH01 and PGT145, are derived from distinct VH genes whose protein products diverge at the amino acid level. The light chains (LCs) of all four antibodies are unique.

Although the unique features of the CDRH3 regions of these Abs are crucial for their neutralizing activities, an important observation made by Andrabi et al. is that VH and VL elements outside of CDRH3 regions are also involved in the neutralizing properties of V2 apex bnAbs, given that engraftment of the CDRH3 regions of PG9 and CAP256.09 onto antibody heavy chains (HCs) derived from non-cognate VH genes leads to loss of neutralizing activity. One of the most intriguing findings in the study is that the inferred germline forms (iGLs) of the bnAbs (reverted to chromosomally encoded sequence, except for the CDRH3 region) displayed neutralizing activities against a subset of the viruses neutralized by the corresponding mutated antibodies. This neutralization was similarly dependent on the glycosylation pattern of Env, suggesting that the modes of neutralization by the iGL antibodies and the mutated antibodies are the same.

The observation that the iGLs can neutralize some HIV-1 viruses is important from the perspective of eliciting V2 apex Abs by vaccination. The finding is particularly interesting because similar studies conducted with bnAbs that target different Env vulnerable sites, such as the CD4-BS or conserved epitopes in V3, revealed that those iGLs do not bind recombinant Env or display HIV-1-neutralizing activities (Hoot et al., 2013; Mouquet et al., 2012). iGLs presumably represent the germline B cell receptors that are expressed on naive B cells and are bnAb progenitors. The fact that the iGL forms of V2 apex bnAbs can neutralize some HIV-1 viruses suggests that specifically designed immunogens that accurately recapitulate the key structural features of the V2 apex could stimulate naive V2 apex bnAb progenitors and initiate the production of these types of antibodies during vaccination.

Although the mutated V2 apex bnAbs neutralize diverse HIV-1 viruses, they display poor reactivity toward soluble gp120 or gp140 recombinant Env proteins derived from these neutralization-sensitive viruses. This is because the epitope recognized by these antibodies has a quaternary nature, which is very difficult to stably reproduce on conventional gp120 or gp140 proteins. Here, by taking advantage of novel approaches to build stable soluble forms of the HIV-1 Env spike (SOSIP) that present quaternary epitopes targeted by bnAbs (Sanders et al., 2013), the authors engineered a SOSIP protein (CRF02_AG) derived from one of the viruses that is neutralized by the iGL forms of PG9, CH01, and CAP256.09. In doing so, the authors have generated the first Env mimic that binds multiple iGL forms of V2 apex bnAbs (Figure 1). This represents an exciting first step toward being able to initiate the process of eliciting V2 apex bnAbs through vaccination.

A caveat of these studies is that the CDRH3 sequences of the mutated Abs were used for engineering the iGL forms. However, according to next-generation sequencing of longitudinal samples from the CAP256 donor (Doria-Rose et al., 2014), the CRF02_AG SOSIP is also recognized by the true progenitor of the CAP256 lineage, which indicates that this immunogen might select for naive B cells with long CDRH3 regions capable of recognizing the V2 apex.

The V2 apex bnAbs are less mutated than some other classes of bnAbs. For example, some CD4-BS-specific bnAbs can be up to up 30% divergent from germline bnAbs (Georgiev et al., 2013). In fact, this study reveals that only a small number of somatic mutations are necessary for achieving significant neutralizing activity. The authors identified eight HC mutations that arose independently in PG9 and CAP256.09. Three of these mutations were also shared by CH01,

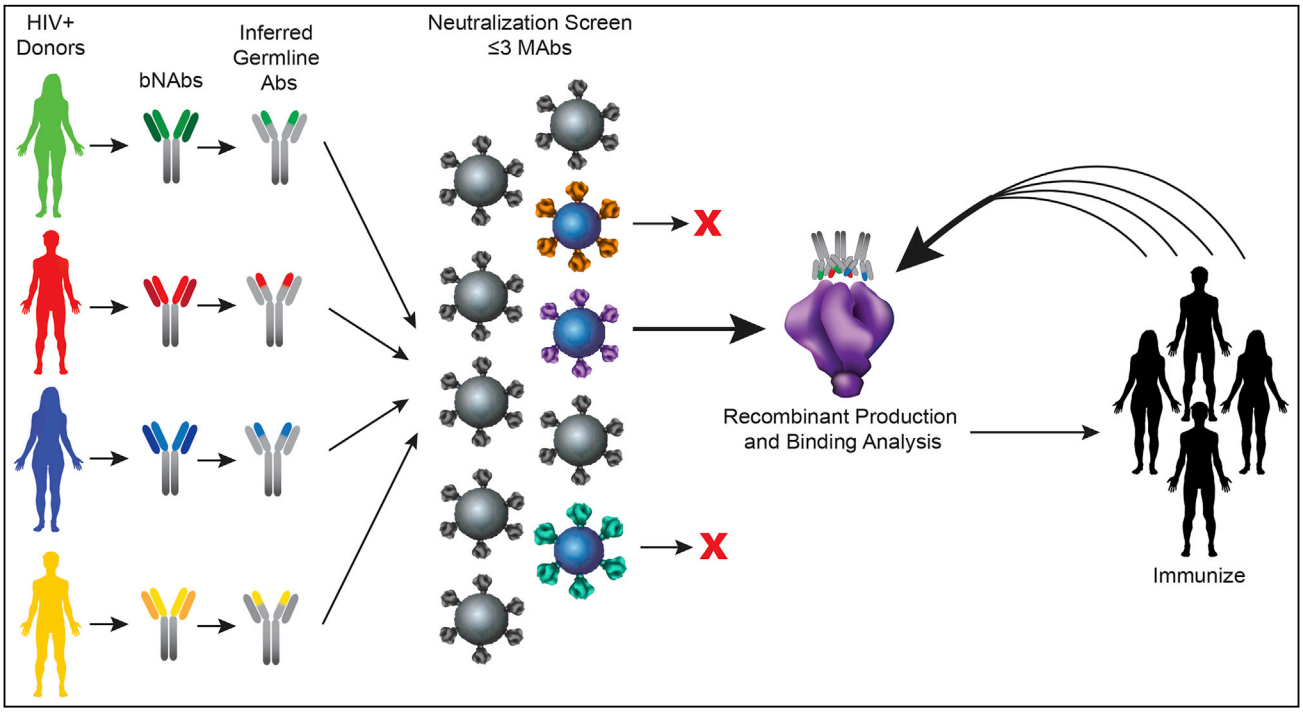


**Figure 1. Selection Process that Andrabi et al. Used to Identify Novel Immunogens Aimed at Eliciting V2 Apex Broadly Neutralizing HIV-1 Antibodies**

Broadly neutralizing antibodies (bnAbs) that target the V1-V2 apex of the HIV-1 envelope protein (Env) were isolated from different HIV-1$^+$ donors. Bioinformatics was used for inferring the amino acid sequences (except for that of CDRH3) of the germline-encoded bnAb progenitors (iGLs). iGL antibodies were tested for neutralizing activity against a panel of diverse HIV-1 viruses. Viruses that were neutralized by the majority of iGL antibodies were selected for recombinant Env production. One Env that successfully recapitulated the structure of the V1-V2 apex was identified by detectable binding to the iGL antibodies. This recombinant Env could be used as an immunogen to stimulate naive B cells expressing B cell receptors that resemble the iGL antibodies targeting the V1-V2 apex.

suggesting that there might be common evolutionary antibody-maturation pathways in these distinct antibody lineages. The introduction of these eight mutations on the HC of iGL PG9 resulted in an expansion in the breadth and potency of neutralization. When eight additional mutations (shared by the LC of the PG9 and PG16 antibodies) were introduced, the breadth and potency of neutralization were further increased to levels approaching those of the fully mutated antibodies. Thus, although extensive somatic hypermutation in both the HC and LC might be required for substantial neutralizing activity in bnAbs that bind the CD4-BS (Dosenovic et al., 2015; Klein et al., 2013), V2 apex bnAbs might be able to achieve significant neutralizing activities with a relatively small number of somatic hypermutations that might be more easily induced with current vaccination protocols.

Finally, it is worth noting the iGL and mutated forms of the V2 apex bnAbs studied here do not display autoreactivity, a property associated with other types of bnAbs and with antibodies with long CDRH3 regions. This could lead to the elimination of bnAb progenitor B cells during their development (Mascola and Haynes, 2013).

In summary, this study provides information that strongly suggests that during chronic HIV-1 infection, Env variants with particular structural features emerge to bind and activate naive B cells expressing B cell receptors with particular CDRH3 regions uniquely poised to penetrate the V1-V2-associated glycan shield at the apex of the Env spike. Furthermore, it is revealed that a relatively small number of somatic hypermutations shared by different V2 apex bnAbs are sufficient for improving the neutralizing activities of these antibodies. The design of a recombinant Env protein that binds the inferred germline forms of V2 apex bnAbs is certainly an exciting advance in the field and will now permit the experimental validation of the immunization regiments proposed in this study to elicit V2 apex bnAbs.

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# Worming Their Way into the Picture: Microbiota Help Helminths Modulate Host Immunity

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**Parasitic helminths are potent regulators of host immunity, including inhibition of allergic inflammation. In this issue of *Immunity*, Zaiss et al. (2015) reveal that microbiota compositional shifts during helminth infection contribute to the multifaceted ways that helminths modulate host immunity.**

The primary goal for a parasite is to remain within their host for as long as possible, while finding ways to propagate. There is little doubt that helminths are successful in achieving this goal, as demonstrated by their ability to mount chronic infections in mammalian hosts. Approximately two billion people worldwide are currently infected with helminths; many infections last for several years, and some even persist for decades. The success of these parasites is attributed to their capacity to modulate host immune responses, which helps their continued colonization and prevents their expulsion. The immunomodulatory capabilities of helminths have a broad scope, extending to suppression of immune responses against foreign antigens and allergens. Thus, helminths or helminth excretory-secretory products have the potential to be used therapeutically in the treatment of allergic diseases (McSorley et al., 2013). Given that helminth infection alters the composition of the bacterial intestinal microbiota (Reynolds et al., 2015) and that the microbiota composition has been implicated in influencing allergic disease (McCoy and Köller, 2015), a major unanswered question is: to what extent does helminth infection reduce allergic inflammation through effects on the microbiota?

In this issue of *Immunity*, Zaiss et al. (2015) present evidence that one pathway by which helminth infection dampens allergic inflammation occurs as a result of compositional shifts within the intestinal microbiota. First, they have shown that infecting mice with the intestinal nematode parasite *Heligmosomoides polygyrus* reduced airway inflammation in a house dust mite (HDM)-induced model of allergy, consistent with previous reports of helminths suppressing allergy (McSorley et al., 2013). Next, Zaiss et al. (2015) reported the striking observation that the helminth-induced protection from airway inflammation is abrogated in mice that had been given antibiotics, suggesting that the intestinal microbiota is required for suppression of airway inflammation. Importantly, antibiotic treatment did not affect *H. polygyrus* numbers. Moreover, co-housing with *H. polygyrus*-infected mice, or transferring cecal contents from *H. polygyrus*-infected mice to uninfected recipients, resulted in protection from HDM-induced inflammation. The authors confirmed that *H. polygyrus* infection was unable to be transferred to recipient mice during these experiments, suggesting that a transferable component of the helminth-modified microbiota was able to provide protection from airway inflammation.

Compositional shifts within the intestinal bacterial microbiota have been previously associated with susceptibility to allergic disease, after alterations in diet or the administration of antibiotics (McCoy and Köller, 2015). How could microbiota shifts during helminth infection contribute to reduced airway inflammation? Zaiss et al. (2015) show that *H. polygyrus*-infected mice, or mice receiving a *H. polygyrus*-modulated microbiota, had elevated cecal amounts of short chain fatty acids (SCFA). They demonstrated that *H. polygyrus* itself was capable of generating the SCFA acetate in in vitro cultures (Zaiss et al., 2015). Indeed, several other helminth species have been shown to produce acetate (Tielens et al., 2010). Because antibiotic-treated *H. polygyrus*-infected mice showed completely abrogated SCFA production, it is likely that the major source of SCFA generated during *H. polygyrus* infection was the bacterial microbiota, rather than the parasites themselves (Zaiss et al., 2015). They further demonstrated that elevated SCFA levels are a